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How to use... lymph node biopsy in paediatrics

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ABSTRACT

Lymphadenopathy is a common finding in children. It often causes anxiety among parents and healthcare professionals because it can be a sign of cancer. There is limited high-quality evidence to guide clinicians as to which children should be referred for lymph node biopsy. The gold standard method for evaluating lymphadenopathy of unknown cause is an excision biopsy. In this Interpretation, we discuss the use of lymph node biopsy in children.

INTRODUCTION

Lymphadenopathy is a common finding in children. It is defined as a palpable lymph node of more than 1 cm in diameter, although some authors will accept as normal lymph nodes in the inguinal region of up to 1.5 cm.^{1 2} These nodes usually arise as a result of infective or inflammatory processes and rarely represent cancer. A thorough history and examination often clarify the cause of lymphadenopathy. However, when the cause is unclear or clinical concerns exist, it may be necessary to obtain a biopsy of the enlarged lymph node to establish the cause of lymphadenopathy. It is important to remember that cancer is rare in children and most enlarged lymph nodes are non-malignant. Yet, missing and, thus, delaying a diagnosis of cancer can have adverse effects, for example, if the cancer progresses to a stage requiring more intense treatment.

PHYSIOLOGICAL BACKGROUND

There are approximately 600 lymph nodes in the human body, which act as filters between the lymphatic and haematological circulations. They consist of structural cells, fibroblasts and immunological cells. The immunological components include cells from the innate immune system (macrophages, dendritic cells and Langerhans cells) and the adaptive immune system (T and B lymphocytes). The cells

are contained within connective stroma and encased in a capsular shell.³

Lymph nodes filter lymph from set anatomical areas and the location of an enlarged node may give a clue as to the site of the underlying pathology (table 1). Lymphadenopathy may occur as a result of infection, inflammation, malignancy, drugs or other disease processes (table 2).

TECHNOLOGICAL BACKGROUND

There are three main options for sampling lymph nodes in children: excision biopsy, fine needle aspiration biopsy (FNA) and core needle biopsy.

The gold standard technique for lymph node biopsy in children is excision biopsy. The reason why it is the gold standard is that it removes the entire lymph nodes and, thus, usually provides sufficient tissue and allows microscopic examination of all regions of the lymph nodes. Tissue obtained by excision biopsy is sent for histological analyses, which comprise morphological assessment, immunohistochemical stains and, in some cases, tests for specific mutations such as translocations or point mutations. In addition, lymph nodes can be disaggregated and analysed by flow cytometry, similar to blood and bone marrow. The tissue can also be sent for microbiological analysis as dictated by clinical assessment. The main disadvantage of excision biopsy is that it is an invasive procedure that requires general anaesthetic and is associated with risk of nerve damage, bleeding, infection, scarring and anaesthetic complications.⁴

Many children referred for excision biopsy do not have cancer. Depending on referral practice and which population is studied, the prevalence of malignancy in children with lymphadenopathy varies from 13% to 33%.⁵ In choosing who to refer for excision biopsy, a difficult balance has to be struck between avoiding unnecessary procedures in some children while not delaying a diagnosis of cancer in others. As outlined in the clinical

Interpretations

Table 1 Summary of sites of lymph node and area drained²

Lymph node site	Area drained
Cervical	Head and neck
Submental and submandibular	Buccal mucosa, cheek and nose
Supraclavicular	Right-sided—thorax, Left-sided—abdominal
Axillary	Ipsilateral arm, breast, neck and thorax
Inguinal	Ipsilateral leg, buttock and lower half of abdominal wall

scenarios, there is limited evidence for using clinical signs and symptoms to preselect patients with a higher likelihood of cancer for excision biopsy. Therefore, FNA and core needle biopsy may be used to triage patient referred for an excision biopsy.

FNA involves the insertion of a fine needle into a mass to aspirate cells. The aspirated cells are expelled onto slides. The needle is then rinsed in saline and specimen sent for cytological investigations, such as morphological assessment, immunophenotyping or microbiological studies.⁶ The procedure has successfully been performed in children under local anaesthetic only without the need for sedation, although many children, in particular young ones, will require sedation or a general anaesthetic.^{7 8} There are few reported complications.⁵ FNA is widely used in adults but has not been adopted in paediatric practice. This is, in part, due to a lack of experience in interpreting the cytomorphological features of malignant cells in children and due to concerns regarding the validity of cytology in evaluating lymph nodes in children.^{8 9} For example, in Hodgkin's disease, the bulk of the enlarged

lymph node is composed of reactive lymphocytes. Only a tiny portion of cells in these nodes are the malignant Reed-Sternberg cells that define Hodgkin's disease. Thus, Reed-Sternberg cells might be easily missed by FNA. Even if captured, it is difficult to interpret a cell outside of the context of its surrounding tissue.

Core needle biopsy has been proposed as a further alternative to excision biopsy in the investigation of lymphadenopathy. It is an ultrasound-guided technique that uses a spring-loaded or automated biopsy gun to obtain specimens. In some children, it can be performed under local anaesthetic. It has fewer surgical complications than excision biopsy. Compared with FNA, it enables a tissue specimen to be obtained. It has also been suggested as a method to assess lymph nodes that are difficult to access.¹⁰

Irrespective of the method used to obtain tissue, when cancer is a possibility, the biopsy should be performed in a paediatric oncology specialist unit, where appropriate oncological, pathological, surgical and radiological expertise is based.¹¹ It should not be underestimated how challenging it can be to process and interpret tissue obtained elsewhere. Certain diagnostic tests require specific storage conditions which are not routinely applied to tissue outside specialist centres.

INDICATIONS AND LIMITATIONS

Should I refer for biopsy every child with a lymph node larger than a certain size (in diameter)?

The degree of lymph node enlargement beyond 1 cm has traditionally been considered to be predictive of malignancy, despite the paucity of high-quality data. The majority of studies that examined the predictive value of lymph node size for malignancy are

Table 2 Summary of causes and mechanisms of lymphadenopathy^{1 2 20}

Cause	Mechanism	Examples
Infection	Cellular proliferation as a result of antigenic stimulus as a result of a local nodal infection or regional infection	Viral—URTI, EBV, CMV, Rubella, Rubeola, VZV, HSV, Coxsackievirus, HIV Bacterial— <i>Staphylococcus aureus</i> , Group A β -haemolytic streptococcus, anaerobes, diphtheria, cat-scratch disease, tuberculosis, non-tuberculous mycobacterium Protozoa—toxoplasmosis
Malignancy	Neoplastic proliferation of inflammatory cells or infiltration of neoplastic cells carried in the lymphatic or haematological circulations	Neuroblastoma, leukaemia, lymphoma, rhabdomyosarcoma
Inflammatory	Immune response to antigen or antibodies	Kawasaki disease, juvenile rheumatoid arthritis, systemic lupus erythematosus, serum sickness, dermatopathic adenopathy
Drugs		Phenytoin, isoniazid, post DTP immunisation
Other		Rosai-Dorfman disease (benign histiocytosis), Kikuchi-Fujimoto disease (necrotising lymphadenitis), storage diseases (infiltration of macrophages filled with metabolite deposits), autoimmune lymphoproliferative syndrome (failure of apoptosis), Castleman's Disease (lymphoproliferative disorder), progressive transformation of germinal centres

CMV, cytomegalovirus; EBV, Epstein-Barr Virus; HSV, Herpes Simplex virus; HIV, Human Immunodeficiency Virus; URTI, upper respiratory tract infection; VZV, Varicella Zoster virus.

retrospective. They were usually based in tertiary centres so are biased towards patients with a higher pretest probability of a cancer and thus cannot be easily applied to the general paediatric population.

There is one prospective observational study¹² of children with lymphadenopathy (n=218) who presented to a Turkish general paediatric clinic. They were followed up for 8 weeks to establish clinical criteria predictive of cancer. The size of the lymph node did not differentiate between children with or without malignancy. A cut-off size of 3 cm had a sensitivity of 66% and a specificity of 80% for malignancy. The authors did, however, find that lymph nodes that increased in size during the follow-up period were more likely to be malignant.¹² It should be noted that there were only six patients in the group with malignancy limiting the validity of these calculations.

In addition, there have been several large retrospective studies of the predictive value of enlarged lymph node size in children referred to tertiary oncology centres. Celenk *et al*¹³ suggest that larger lymph nodes are more likely to be malignant (OR 1.45; 95% CI for OR 1.02 to 2.04) but do not provide a size cut-off for decision-making.¹³ Oguz *et al*¹⁴ show that a significantly higher proportion of malignant nodes were greater than 3 cm in diameter, but stopped short of determining the precision of lymph node size in identifying cancer. They also found that children with lymph nodes enlarged to 1–3 cm were more likely to have a benign process, although around 14% of children with cancer had a lymph node size in this range. This was particularly true if the lymph node was in the supraclavicular region.¹⁴ This finding has been supported by other authors.^{4,5}

Overall, at present there is insufficient evidence to base a decision for referral for biopsy on lymph node size alone, although it seems that larger nodes may be more likely to be malignant, particularly if they are increasing in size. Enlarged supraclavicular nodes should always raise suspicion.

Should I refer for biopsy every child with lymphadenopathy persisting for more than 4 weeks?

Another clinical feature of lymphadenopathy traditionally considered to be a red flag for cancer is duration of enlargement. The underlying evidence base is conflicting, with most studies using an arbitrary cut-off of 4 weeks.

In their prospective observational study of 218 children with lymphadenopathy referred to a general paediatric clinic, Bozlac *et al*¹² found no significant difference between benign and malignant groups in terms of duration of lymphadenopathy.¹² This finding was reflected in the systematic review of Locke *et al*⁵ who showed no relationship between duration of malignancy and likelihood of serious pathology, with some studies showing a lower rate of malignancy in patients in whom lymphadenopathy was present for a prolonged period of time.⁵ We would, however,

caution against interpreting long persistence of lymph node enlargement as reassuring. There are well-described childhood lymphomas that follow a more indolent course presenting with enlarged lymph nodes that have persisted for long periods, 12 months or more, without progression in size or development of other symptoms.¹⁵

In contrast to the general paediatric population, when looking at children assessed in specialist paediatric oncology unit retrospectively, duration of symptoms was significantly associated with cancer. Oguz *et al*¹⁴ report that among patients with a malignant process, 96.4% had chronic lymphadenopathy, representing 44.8% of the patients referred with chronic lymphadenopathy.¹⁴

Taken together, therefore, the notion that duration of lymphadenopathy is a red flag for cancer in children may, or may not be, tenable. In the setting of a specialist unit where the prevalence of cancer is higher, persistence of enlargement should clearly be a worrying feature. Whether this is the case in general paediatric practice remains unclear. Therefore, in the absence of convincing evidence to the contrary, we would recommend to general paediatricians to continue to regard the persistence of lymphadenopathy as a red flag for cancer.

Does a negative fine needle aspiration biopsy exclude cancer?

FNA has been proposed as a screening test in children with lymphadenopathy to reduce the need for excision biopsy. It is hypothesised that by performing an initial FNA, it may be possible to reduce the need for excision biopsy.

In a recent systematic review, Locke *et al*⁵ found 12 papers looking at the use of FNA in the investigation of head and neck masses and lymphadenopathy in all sites in children. The studies were retrospective with sample sizes varying from 29 to 288 patients. Overall, FNA had a specificity of 92%–100% for diagnosing cancer and a sensitivity of 67%–100%.⁵ Since Locke's review, further studies have examined the performance of FNA. In the largest of these, FNA was evaluated in 217 children biopsied in a tertiary centre. The authors calculated that the sensitivity of FNA was 92% with a specificity of 100%.⁸ Two cases of lymphoma had been missed and misdiagnosed as reactive lymphadenitis.

Overall, it may be the case that FNA is a valid tool in evaluating lymphadenopathy in children. From the available data, it seems that when a cell is aspirated that looks malignant, cancer is a very likely diagnosis. However, FNA has a variable performance in excluding cancer. As a result, if there are clinical concerns such as progressive growth of lymph node or systemic symptoms suggesting a malignancy, an excision biopsy should be arranged to confirm diagnosis.

Does a negative core needle biopsy exclude cancer?

Core biopsy has also been proposed as an alternative to excision biopsy for lymphadenopathy. Few studies have examined the issue specifically in children.

Bain *et al*¹⁶ examined the use of core biopsy in 12 children with lymphadenopathy. They found three cases of cancer at core biopsy, which were confirmed at excision biopsy. The authors note that no additional information was obtained at open biopsy in comparison to core biopsy. They did not report whether any of the patients with negative core biopsy were later diagnosed with cancer.¹⁶ Ehrlich *et al*¹⁷ studied the diagnostic precision and adverse events of core needle biopsy, FNA and excision biopsy in 185 children with Hodgkin's lymphoma. Of these, five underwent core biopsy. In four of five of these, Hodgkin's disease was identified.¹⁷ Additional insight into the utility of core biopsies may be derived from previous studies of mixed adult and paediatric populations.^{18 19} However, as outcomes for children and adults have not been differentiated in these reports, the data are difficult to interpret in our context.

Taken together, there really is insufficient evidence to answer our question. Whether or not core needles biopsy is an adequate tool for evaluation of FNA in children remains to be established.

Topics for further research

Most importantly, sufficiently powered prospective research is required that identifies clinical features, that is, signs and symptoms, that can be integrated into robust algorithms that allow primary and secondary care providers to decide whom to refer for a specialist opinion. Given the overall low prevalence of cancer in children with lymphadenopathy in the general paediatric population, such studies would probably require a long-term, regional, if not national, effort.

Regarding FNA, it would be important in future studies to measure clinically important outcomes, not just sensitivity and specificity of FNA. In particular, we need to know whether it actually matters for patient outcome that occasionally diagnoses of cancer are missed by FNA and thus delayed.

As for core needle biopsy, an important consideration for future studies will be whether the sufficient amounts of tissue can routinely be obtained to perform emerging diagnostic tests on tissue, such as next-generation sequencing of cancer DNA.

Finally, it may be useful to look at the experiences of children (and their parents) who have undergone the various biopsy procedures to establish their preferences.

Clinical bottom line

- ▶ Lymphadenopathy in children is common and cancer is rare.
- ▶ Although detailed history and examination establish a trivial diagnosis in most cases, cancer should be considered in children with lymphadenopathy.

- ▶ The decision to refer a child for lymph node biopsy cannot be made by consideration of size or duration of lymphadenopathy.
- ▶ Excision biopsy remains the gold standard technique for sampling of enlarged lymph nodes.
- ▶ Biopsies should only be performed in specialist paediatric oncology centres multidisciplinary team.¹¹
- ▶ If you do suspect cancer, pick up the phone and speak to a paediatric oncologist.¹¹

Test your knowledge

A 5-year-old child is referred by the general practitioner with a 2-week history of swelling to the left side of her neck. Examination reveals a firm mobile mass in the anterior cervical chain measuring 2 cm by 2 cm. Diagnosis of lymphadenitis is made, and the child is commenced on antibiotic therapy. On return for review 1 week later, the swelling has increased in size and is now measuring 3 cm by 4 cm.

1. Which of the following statements are true?
 - A. Further referral for biopsy should not be made until child has completed the course of antibiotics.
 - B. Increasing size of lymph node on antibiotic therapy should trigger clinician to refer for urgent biopsy,

You discuss the case with the local ENT team who suggest FNA to rule out malignancy

2. Which of the following statements are true?
 - A. An FNA showing reactive lymphadenitis rules out malignancy.
 - B. FNA can be performed without the need for a general anaesthetic in cooperative children.
 - C. An FNAB revealing malignant cells is sufficient for diagnosis of malignancy and further investigation, and classification is not required prior to commencing treatment.

You are referred a 14-year-old patient with generalised lymphadenopathy. Examination reveals generalised lymphadenopathy and mild hepatosplenomegaly.

3. Which of the following statements are true?
 - A. The presence of an enlarged supraclavicular lymph node should trigger clinician to refer for urgent biopsy.
 - B. In view of a clinical picture, clinician should always wait for EBV serology prior to arranging further referral or investigations.
 - C. A clinician should have a lower threshold to refer patient 2 for a biopsy, rather than patient 1 due to the age of patient.

The answers are after the references.

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Answers to the multiple choice questions

- 1A. false
- 1B. true
- 2A. false
- 2B. true
- 2C. false
- 3A. true
- 3B. false
- 3C. true