Is Flow Cytometry helpful in diagnosing lymphoma in patients undergoing percutaneous nodal biopsy?

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Background

Flow cytometry has been used for a number of years in assessment of lymphoma in fresh lymph node specimens. It gives an accurate, rapid assessment of disease and is used in conjunction with histology to assess grade and type of lymphoma.

In University Hospital Bristol we have moved away from open biopsy of nodes in patients with suspected lymphoma due to surgical delays and morbidity associated with open procedures, and now carry out core biopsies on over 90% of patients with assessable lymphadenopathy.

We ran a pilot study to look at flow cytometry assessment of nodes via FNA in these patients to assess if the this technique was helpful in the diagnosis of lymphoma.

Sample quality- Viability

Disaggregation may result in cell death

Tissue may be necrotic

Viability test always carried out by flow lab prior to immunophenotyping analysis:

- Non-specific antibody/fluorochrome binding by dead/dying cells
- Propidium iodide used to check viability
  - Cannot permeate intact cell membranes
  - Intercalates DNA (nucleic acids)

Flow cytometry

A flow cytometer analyses individual cells as they flow in a liquid medium through a laser beam.

The particles are sorted by size, relative granularity, cell complexity and fluorescence.

Light scattering at different angles can distinguish differences in size and internal complexity, whereas light emitted from fluorescently labelled antibodies can identify a wide array of cell surface and cytoplasmic antigens.

Method

Patients undergoing core biopsy and FNA for suspected lymphoma were consented to have a further FNA sample taken for flow cytometry studies. Cytometry sample taken with a 22G spinal needle into the node with 3-4 passes through the cortex of the node. Aspirate washed out in 5 mls of skin biopsy medium (from Bristol Cytogenetics lab). Aspirate stored at 4 deg in a fridge until assessed on a LRS Fortessa 10+ colour Flow Cytometer.

Results

We assessed samples from axillary, cervical and inguinal nodes from Nov 2014 to Jan 2015.

80 samples in all from 22 patients. Most had cores/ flow and cytology. Some had repeat cores with a few needing lymph node excision. 91% of patients had diagnosis from percutaneous biopsy only, negating need for open procedures in most cases.

Of the 22 patients 15 had lymphoma/ CLL. 2 had reactive nodes. 1 metastatic lung Ca 1 necrotic node 2 infection (inc TB) 1 unsatisfactory sample

Discussion

We have found flow cytometry a helpful adjunct for diagnosis of lymphoma.

No false positives.

If initially patients have an FNA and flow for 3 metastatic disease or lymphoma, if flow positive for lymphoma they can be recalled for cores.

Quick turn around time for flow cytometry of 24-48 hours which speeds diagnosis especially of high grade disease.

Flow adds to the confidence of the reporting by specialist pathologists of type of lymphoma.

Due to the results of the pilot study we now routinely use flow cytometry for all our lymph node samples for possible lymphoma.

Correlation

Flow cytometry picked up all high grade disease except 1 Hodgkin’s disease case.

Flow known not to detect Hodgkin’s disease due to the large cell size, but this is readily picked up on cytology and core.

10/11 other lymphomas detected on flow and cores and were concordant.

One low grade lymphoma reported as reactive on flow.

No false positives on flow.

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